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Research Article

Performance of selected HIV testing centers in a HIV Proficiency Testing Scheme in Kenya: a case study

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Background: The Proficiency Testing (PT) for Human Immunodeficiency Virus (HIV) using Lateral flow assays provides an avenue for participating institutions/individuals to assess their technical competence in testing for HIV using LFAs that are recommended in the National HIV Testing Algorithm (NHTA) in Kenya. It also provides confidence to the participating institutions and potential users of their services besides giving the institutions an opportunity for improvement.

Objective: To determine the performance of selected HIV testing centers in a HIV PT Scheme in Kenya

Methods: Fifty one participants (51) in Kenya were selected from 7 sites (Kisumu, Mombasa, Kilifi, Nairobi and Malindi) to participate in this PT round. The sites comprised both private sector and institutions that do not participate in the National HIV referral Lab-PT scheme. They were provided with panels containing six samples to analyze using the current NHTA in Kenya. Obtained results were sent to our laboratory electronically.

Results: Eighty nine percent (89.0%) of the panels were correctly identified by the participants as positive or negative. Of the 11.0% errors, 74.2% were committed in one or more test result obtained while 12.9% committed in failure to follow NHTA. Two minor errors repeated by participants were; failure to record the final results in spite of obtaining correct tests and correct reactive results with the first and second test kits but in conclusion the participant recorded negative (12.9%). Root cause analysis revealed that the error committed by participants were as a result of failure to observe the kit manufactures' instructions and NHTA guidelines.

Conclusion: The results of this PT Scheme enhance the need for constant training of personnel conducting HIV testing and Counseling in Kenya on proper techniques of carrying out HIV testing using Lateral flow assays in the NHTA.

Key words: HIV, Proficiency Testing, errors, false negative, false positive.

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1. Introduction

It is important that a HIV Testing Centers (HTC) and individual testers show competence in performing all HIV tests through External Quality Assessment (EQA)/Proficiency Testing (PT), on-site audit or blinded rechecking involving re-testing of 5-10% of negative samples and the positive samples at reference laboratory (WHO-Rapid HIV Tests Guidelines 2004). The PT for HIV Lateral flow assays (LFAs) provides an avenue for participating institution to assess their technical competence in testing for HIV using LFAs that are recommended in National HIV Testing Algorithm (NHTA). It provides assurance on participating laboratories/ and potential users of these laboratories' services with respect to precision, accuracy and sensitivity of detection.

The scheme is not designed to evaluate the total operational competence of a laboratory on the basis of a set of predetermined criteria (Singapore National HIV Reference Laboratory Department of Pathology 2012).

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LFA PT Scheme were introduced in Kenya in the year 2007 by the National HIV Reference Laboratory (NHRL). This laboratory carries out Dried Tube Specimen (DTS)-based panels which are distributed three times per year to the testing sites enrolled in the LFA PT Scheme. Before 2011, site staff performed PT panels testing in group or testing was done by the most competent tester within the testing site. From 2011 up to now DTS panels have been distributed to individual HIV testers to assess their performance. Poor performing testers are targeted with quality intervention (Kitheka, 2012). In Kenya the LFA-PT activity carried out by NHRL between March and May 2012 among 365 participants drawn from 289 facilities reported that 11.5% of the participants could not test all the panels correctly (WHO-Rapid HIV Tests Guidelines 2004, Kitheka, 2012). The program did a Root-cause analysis and established the following causes of failure: failure to follow approved NHTA (23%); lack of job aides (19%) in the facilities, lack of timers hence incorrect test timing, failure to adhere to written procedures, use of wrong sample volume, use of wrong test reagent and non-troubleshooting of invalid results (Kitheka, 2012).

EQA Scheme in a NHRL in South India carried out in 2012 showed that 97.13% reported correct results (Sushi, 2012). Livinus et al (2011) reported concordance of 82.0% in a pilot PT activity in Nigeria by using Dry Blood Spot (DBS) (Livinus et al, 2011). To complete a HIV test event successfully in PT scheme a participating laboratory is often required to achieve a score of at least 80% for Diagnostic Services or 100% for Donor Services. Failure of the testing program is defined as failing two of three consecutive test events (Wadsworth Centre, 2013).

Various studies have shown that some laboratory staff experience difficulties in interpreting positive results that are close to being indeterminate where the accuracy of interpretation of 80-97% has been reported (Learmonth et al, 2008).

This study aimed at determining the performance of selected HTCs in a HIV PT Scheme in Kenya. This study included assessing of both the testing facility and individual performance of laboratory staff and to provide analytical information to participating laboratories for self-evaluation. This study was also carried out as part of a wider project to develop the capacity of the Kenya Medical research Institute (KEMRI) Production facility as the Centre of Excellence in production of various quality PT materials for the East African region.

2. Methodology

2.1 Number of participants and panels

Fifty one participants (51) were selected from 7 sites: (KSM-1 and KSM-2 in Kisumu, MSA-1 and MSA-2 in Mombasa, KFI-1 in Kilifi, NBI-1 in Nairobi, MAL-1 and MAL-2 in Malindi) participated in this PT Round. The participants were those regularly involved in HIV screening from private sector and public institutions that do not participate in the NHRL-PT Scheme. The participants received a set of six (6) PT panels each round at an interval of four (4) months. Each time the participants received the panel was referred to as a PT round, and three rounds made up a PT program.

In each PT round, panels from each pool were given a unique number (alphanumerical digit) that is only known to personnel in charge of the PT program for traceability and to avoid collusion of the results among the participants.

2.2 Preparation of HIV DTS Panels

HIV PT panels were prepared from serum (both HIV positive and HIV negative) obtained by re-calcification of the plasma harvested from waste blood units from Blood Transfusion Centers in Kenya as per the procedure described. Briefly, the plasma units were tested for Hepatitis B Virus (HBV) and HIV. All HBV positive units were discarded while retaining and distinguishing all the HBV negative units as being either HIV positive or negative. For the re-calcification of plasma, calcium chloride solution was added to each plasma unit and the mixture incubated in a water bath at 37°C for an hour. After forming a clot the mixture was placed overnight in a freezer at -20°C, and then removed. Then, serum solution filtered and heat inactivated. A green dye was added to the serum and dispensed into labeled serum tubes and left open in a safety cabinet to dry overnight (12 hours). The tubes were then capped and affixed appropriately with coded labels.

Homogeneity Assessment of the Panel sera

After labeling the panels 10% of them were randomly picked per pool and tested using the LFAs used by the participants at that period, to confirm their HIV status. The results obtained from this test were compared with the expected qualitative results of Enzyme-Linked Immunosorbent Assays (ELISA).

External QC of the materials

Panel sera from each of the six pools were sent to an ISO 15189 accredited laboratory (KEMRI-CDC Kisumu) for external Quality Control and their status confirmed as concurrence.

Shipping of the PT materials

The DTS were transported to the selected site by the PT providing personnel. The content of the shipment included the six panel sera, instruction note and Delivery note. During this time the participants were trained on how to minimize the obvious errors in PT. The participants were also allowed to ask questions that bothered them.

2.3 Sample Testing Procedure

In each of the PT round the participants were to test the PT panels using the available test kit according to the NHTA. Each participant was expected to use a set of up to three sets of kits to identify the qualitative status of each panel. Therefore if a sample was non-reactive in the first kit the participant was to fill non-reactive in the form provided. Similarly if a sample was reactive in the first kit the participant was to test the same sample using the second kit and if it remains reactive he/she

was to conclude as reactive in the form. On the other hand a reactive sample on only the first kit with the second kit being non-reactive the participant was to be conclude as indeterminate (this option was only for those participants without the third test kit). The status of a sample that was reactive on the first kit but non-reactive on the second kit, was to be determined by a third kit. The result of any two agreeable results out of the three test kits was considered the HIV status of the sample. Table 1 shows the test kits used during the PT program.

2.4 Data management

The data forms filled by each of the participating staff were collected by the supervisor and emailed to KEMRI and entered into one database, using the Microsoft Excel program and Statistical package for social scientist (SPSS). The preliminary data entry was reviewed, and double checked through a data cleaning process by PT Providing Team before informing the participants of their performance. Thereafter the results were analyzed. There were six types of errors assessed in this PT exercise namely:

ETR (Error in Test kit Result): One or more individual test kit result obtained were wrong, and hence wrong conclusion

EBC (Error with unclear Basis of Conclusion): Made correct conclusion on two positive samples, despite reporting the third test kit result as "invalid"

EOC (Error of Omitted Conclusion): correct individual test kit results were obtained but conclusion was omitted

ECI-(Error in following Coding Instructions): correct test kit results and conclusion but had not used the provided codes, "Reactive" or "Non-reactive", instead reported each test kit result as "positive" or "negative"

ENE (Error No test kit result entered on the form).

EAU (Error on Algorithm Used): the participant used a wrong algorithm during the testing, starting with a different test kit other than determine kit.

The three errors were regarded to be of lesser gravity; errors associated with ECI, EAU and EOC results because the staff committed them after obtaining a correct individual HIV test kit results. For this reason we disaggregate these three categories of errors in our results presentation.

Performance of the participant or the institution refers to the proportion of samples correctly tested and reported.

2.5 Ethical considerations

The ethical approval was given by KEMRI Scientific Steering Committee (Ref.: **SSC No. 2190**) and each participating lab filled a consent form before participating in the PT program.

3. Results

3.1 Participants profiling

In all the three rounds, a total of 51 different individual participated in the PT program from 11 facilities. In the first, second and third rounds, there were 41, 18 and 16 participants respectively and each received a set of six panel for each round.

Majority of the participants were Laboratory technologists (94.3%) and the rest were supervisors and counselors; 3.5% and 1.9% respectively.

In the first two PT rounds Determine™ HIV-1/2 kit was the first test kit followed by Uni-Gold™ HIV test, however in the last round the NHTA changed; health workers were to first test the samples using KHB Colloidal Gold and all reactive samples were to be confirmed using at least 1(one) or more other kits that included either first response and KHB Colloidal Gold.

The third PT round was performed when the transition was in progress and there were therefore a number of test kits used based on availability. **Table 1** shows the list of the kits that the participants used during the PT Rounds. Majority of the participants had KHB Colloidal Gold and First Response but there was scarcity of third test kit as observed in all facilities except two.

Table 1: Test Kits and their status

PT Round	HIV test kits used			Status of panels distributed		Total number of panels distributed	
	1st kit	2nd kit	3rd kit*	Positive	Negative	Panel	Participants
Round 1	Determine™ HIV-1/2	SD Bioline	Uni-Gold™ HIV test	82	82	246	41
Round 2	Determine™ HIV-1/2	Uni-Gold™ HIV test	Uni-Gold™ HIV test	36	36	108	18
Round 3	KHB Colloidal Gold/ Determine™ HIV-1/2	First Response™	Uni-Gold™ HIV test	32	32	48	16

** Some labs did not have the third test kit*

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Table 2. Performance of various HIV panels

PT Round	Panels	Number of Panels without any error (%)	Panels with an error
Round 1	Positive (n=123)	99 (80.4%)	24 (19.6%)
	Negative (n=123)	120 (97.6%)	3(2.4%)
	Total (n=246)	219(89.0%)	27(10.9%)
Round 2	positive (n=54)	50(92.6%)	4(7.4%)
	Negative (n=54)	48(88.9%)	6(11.1%)
	Total (n=108)	98(90.7%)	10(9.3%)
Round 3	Positive (n=60)	53(88.3%)	7(11.7%)
	Negative (n=30)	26(86.6%)	4((13.3%)
	Total (n=90)	78(87.8%)	11(12.2%)

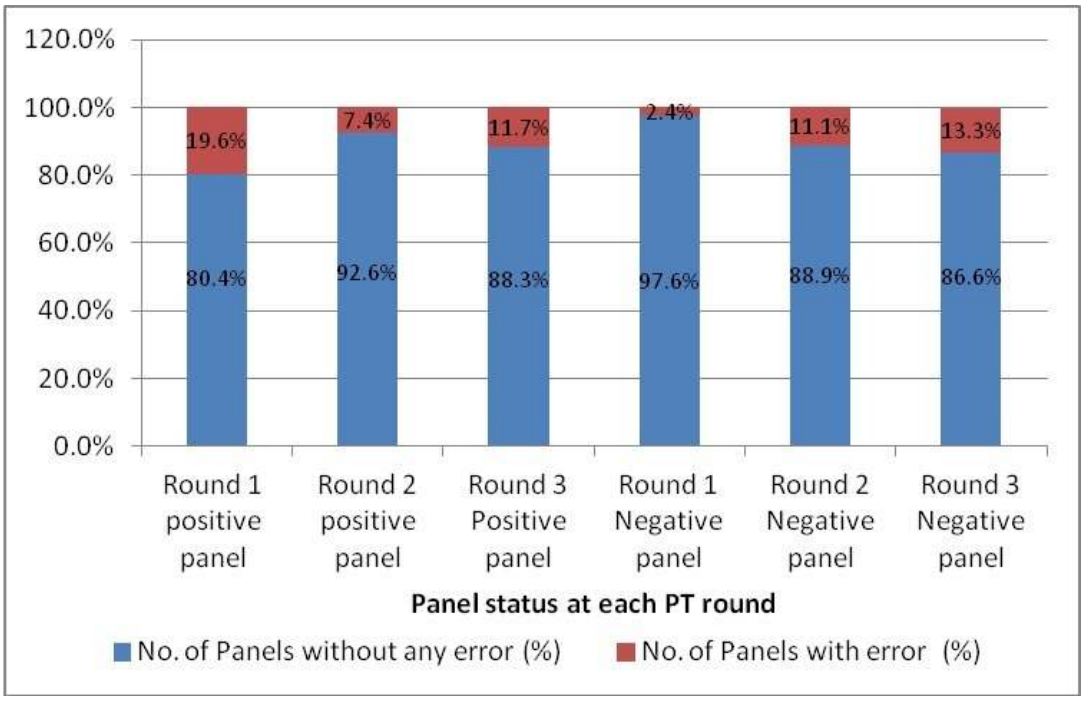

Figure. 1: Performance of the participants on each panel PT round

3.2 Performance of the participants

The average performance of the participants in the three rounds was 90.6%; in which the first round the overall performance was 89.0%, it then rose to 90.7% in the second round and finally 92.3% in the last round (**Table 2**).

3.3 Performance on each category of HIV panels

The PT panels were qualitative with either positive or negative results in all the three rounds.

The number of positive panels correctly tested without any error in round one was 80.4% and in round 2 it increased to 92.6% and then dropped to 88.3% (**Table 2**). The overall performance when testing positive panels was 87.1%. In the negative panels, the first round has the highest number of panels tested without any error, this number gradually dropped to 86.6% in the third round (**Figure 1**). The overall performance for the negative panels was 91.0%, making it easier for the participant to correctly test the negative panels compared to the positive panels.

3.4 Errors made by the participants

There was observed an indirect proportionality between the errors and the PT rounds in that the number of errors decreased from five in round one to two different major errors in round three. On the other hand, there were ten different types of error made by the participants in the three rounds as listed in **Table 3**. In each round a set of new errors different from the previous one were observed such that in round two the participant had errors in conclusion and in round three the three types of errors (wrong application of algorithm, stated and type of test kits used not stated) were on the application of the test kits.

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Table 3: Errors made by participants in each PT round

Performance/Errors made	PT round 1 Status of panel			PT round 2 Status of panel			PT round 3 Status of panel		
	Pos	Neg	Total	Pos	Neg	Total	Pos	Neg	Total
No error made	99	120	219	50	48	98	52	26	78
Errors made									
Error in the first test (determine)	1	-	1	-	1	1	-	-	-
Error in second kit (Unigold) resulting in Indeterminate results	13	-	13	2	-	2	-	-	-
error in second kit (Unigold) wrong conclusion	9	-	9	-	-	-	-	-	-
Got correct kit Results but made wrong conclusion	1	-	1	-	1	1	-	-	-
Got correct results but no test kit results Entered	-	3	3	1	3	4	-	-	-
Got correct kit Results but made wrong conclusion	-	-	-	-	1	1	-	-	-
wrote correct conclusion despite of error in test results	-	-	-	-	1	1	-	-	-
wrong application of algorithm (Unigold kit as first Kit)	-	-	-	-	-	-	4	2	6
False positive, and the kit used not stated	-	-	-	-	-	-	-	1	1
Type of test kits used not stated	-	-	-	-	-	-	4	1	5
Total	123	123	246	54	54	108	60	30	90

Table 4: Errors Performed by the participating Institutions

Facility	Error performed by the participant /panel sera		
	No error n (%)	Had an Error n (%)	Total
NBI-1	24 (100.0%)	-	24
MAL-1	6 (100.0%)	-	6
KFI-1	18 (100.0%)	-	18
MSA-2	12 (100.0%)	-	12
MAL-2	2 (33.3%)	4 (66.7%)	6
KSM-1	139 (92.7%)	11(7.3%)	150
MSA-1	18 (60.0%)	12 (40.0%)	30
Total	219 (89.0%)	27(11.0%)	246

3.5 Errors in PT Round 1

In round one of the PT program, out of 246 panels distributed with an equal number of positive and negatives, a higher number of errors were observed among the positive panel; 4/5 (80.0%) as compared to the negative panels. There were 24 errors observed among the positives and three (3) among the negatives. Three out of the five (60%) errors committed were related to particular test kit, in which second test kit (UniGold) gave false negative results in 22/24 (91.7%) errors, and determine test kit gave 1/24 (4.2%) false negative results.

The participants differed in how to conclude an ETR (Error in one Test kit Result) with one positive and the

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other negative. More than half (59.1%) correctly concluded as indeterminate results and the rest concluded as negative results without using a tie breaker. Among the negative panels, one participant got correct results in each test but failed to record test kit conclusions.

3.6 Errors in PT Round 2 and 3

Round two had similar errors as round one but with lower frequencies (**Table 3**). The only different errors observed were on conclusion whereby the participant got correct kit Results but made wrong conclusion and the other wrote correct conclusion despite of error in test results.

The type and number of errors in round three decreased to only two from five that were observed in round two. Similarly the types of error shifted from kit related to personal and application related errors. The two errors done by the participants were wrong application of the national algorithm whereby the participant started with Unigold test kit and a participant failing to record the type of test kits they used (**Table 3**).

3.7 Institutional Performance and reproducibility

In Round 1, participants from institution MAL-2, KFI-1, NBI-1, and MSA-2 performed no error in any of the panel sera; they scored 100% in all the tests. MAL-2 and MSA-1 and KSM-1 committed 66.7%, 40.0% and 7.3% errors respectively (**Table 4**). Out of the 27/246 (11.0%) errors, 44.4% were from MSA-1, 40.7% from KSM-1 and 14.8% were performed by MAL-1. The errors performed by each institution were inter-related among the participants.

3.8 Corrective actions taken to improve the errors observed

After every round the PT providing team analyzed the data and sent back the result to the participant/institutions indicating the expected results and what the participant got. This was followed by training the participants on the common errors observed during the PTs. Through those trainings, it was realized that the participants who got indeterminate results never strictly followed the kit manufacturer's user manuals especially on the time to read the results. Except on the new PT participants the error was eliminated by the third round of the PT (**Table 3**).

4. Discussion

HIV testing is a key component in control and management of the Virus. With the introduction of point-of-care testing technologies commonly referred as Voluntary Counseling and Testing (VCT) centers and sometimes involving the use of non-laboratory staff in routine testing has further increased the complexity of Quality Assurance (Alemnji et al, 2011).

There are two acceptable HIV diagnostic testing strategies: serial strategy or parallel strategy that includes two or more tests (Yan et al, 2014). Kenya being a high HIV prevalence country, parallel testing is the most acceptable option. During the time of administering the PT (mid 2014), the Kenyan NHTA used Determine™ HIV-1/2 (Abbott Diagnostic Division, Hoofddorp, The Netherlands), as the first kit followed by Uni-Gold™ HIV test (Trinity Biotech, Oregon, USA) as the second kit.

Eighty nine percent (89.0%) of the DTS panels were correctly detected by the participants as clear positive or negative, whereas 11.0% had an error in detection using any of the two kits or applying the NHTA requirement. These results were very close to those found by the NHRL (Kenya) in the DTS-based HIV PT in 2012 in which the rate of errors was found to be 11.5% (Kitheka, 2012). Better results have been obtained in the PT Program in developing countries such as India where the rates of detection of right results has been 97.13% (Wadsworth Centre, 2013). There was progressive reduction of the errors obtained by the participants with increase in the number of participation in the PT program. This was initiated by training and constantly reminding the participants as part of the PT package. Through this model, a number of reasons for the errors obtained emerged in our root cause analysis. The main cause of error was improper application of the kit user manual especially on the waiting time. The participants read the results before the required time. In round three there was a drastic reduction in the number of mistakes performed by the participants. From the participants' results, mistakes performed were of lesser gravity with only one mistake of heavier gravity. More often than not, the lesser gravity mistakes do not necessarily lead to wrong screening of the sample; the mistakes usually violate the current HIV screening guidelines

The most common errors performed by the participants in the three rounds were ETR (Error in one or more test kit resulting in indeterminate results. The second test kit gave more false negative results than was later found to be due to human errors. Conclusion of an indeterminate result was a challenge and some participants preferred concluding results as negative even without using a third tie breaker kit. Such error was later observed to be due to lack of structured referral system of any HIV controversial result within these facilities. Other common mistakes observed included inconsistence in reporting of results; one participant did not record the final staff results despite obtaining correct tests while another participant got correct reactive results in the first and second test kits but in conclusion the participant recorded negative.

5. Conclusion

There is a need for constant training of all the HIV point of care providers on HIV testing. Our results and successes as PT providers were as a result of close monitoring and training of the participants during and after the PT round. Such training and proper communication encouraged participants on how to improve their performance as it was observed at every PT round. The type of errors also shifted from major errors like obtaining false test kit results to minor operational errors such as failure to record test kits used in the third round. Therefore, a careful approach towards improvement of participants and laboratories that encourages best practices, and review of

government policies in point-of-care testing is needed to improve quality of testing as decentralization takes place (Alemnji et al, 2011).

Conflict of Interest Declaration

The authors declare no conflict of interest.

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